

FLSC Standard Operating Procedure for Sterile Harvest of Organs in Rodents

Purpose

It is necessary to maintain sterility when organ harvest is done for the collection tissues for cell culture or for transplantation. To ensure sterile tissues or organs the following procedures have been formulated.

Materials

Sterile instruments for the skin – iris scissors, thumb forceps	
Sterile instruments for tissue/organ harvest – iris scissors, thumb forceps	
Absorbent Benchcote® or paper towels	Styrofoam board
Sterile surgeon's gloves	Sterile petri dish
Sterile media	Sterile saline or PBS
70% alcohol in a squirt bottle	Sterile 25- 20 gauge needles

Preparation

1. Alcohol the interior of a biosafety cabinet and allow the cabinet to run with the blower on for at least 15 minutes. If the hood is equipped with UV sterilization lamp it can be run prior to initiating the harvests.
2. Apply personal protective equipment including: mask, bouffant, sterile surgical gown or clean observation gown. Make sure the gown covers the wrists to prevent the shedding of skin cells into the hood interior, opened body cavity, petri dishes, or sterile media.
3. Animals will need to be euthanized just prior to the tissue harvest, especially if there will be DNA or RNA assays done. This will necessitate two persons working for this procedure – one person (non-sterile) to euthanize the animals and another (sterile) to perform the sterile harvests.
4. Put on clean gloves for the setup of the hood.
5. Load equipment into the hood and arrange it to allow a unidirectional work flow. This will protect the harvested tissues or organs from accidental contamination from the animal carcasses.
6. Open the sterile instruments respecting the sterile interior of the packages.
7. Place the absorbent Benchcote® or paper towels into the hood on top the Styrofoam platform and wet with alcohol.
8. The non-sterile person will euthanize the animals.

Procedure

1. Double glove using sterile surgical gloves.
2. Have the non-sterile person place the carcass on the absorbent paper and completely soak the carcass with 70% alcohol.
3. Using the skin instruments, make a horizontal cut in the skin just anterior to the prepuce (in males) or the pelvic bone (in females). Take care not to cut through the abdominal musculature.
4. Set the skin instruments aside.
5. Place the thumb under the cut and grasp the cranial edge of the cut. Using the other hand securely hold the hind limbs and tail while pulling the skin toward the head.
6. The skin will tear away from the body just above the hind limbs and can be turned inside out over the head and forelegs. This effectively removes hair contaminants during organ harvest.
7. Soak the body again with 70% alcohol and remove the top gloves which are no longer sterile.
8. When harvesting subcutaneous tissue, the skin is not stripped from the body.

Mammary gland harvest:

Instead the skin is incised from the pelvis to the top of the manubrium sternum along the midline using the skin instruments. Lateral cuts are made at the hind limbs and axillary areas. Using the forceps lift the edges of the skin and affix the margins to the Styrofoam board using the sterile needles. Once the skin has been reflected away from the body, the skin instruments are set aside. Soak the exposed surfaces with 70% alcohol and begin the harvest of tissues using a new set of sterile instruments. The harvested tissue can be rinsed with sterile saline prior to placing in culture media.

Subcutaneous tumor harvest:

The body is positioned to expose the area of interest. The skin is cut along one side of the tumor using the skin instruments. Carefully reflect the skin to expose the tumor. Place the skin instruments to the side and soak the exposed tissues with 70% alcohol. With new sterile instruments, dissect the mass from the underlying attachments and sever blood vessels feeding the mass. The tumor is then placed in culture media. It is imperative that sterile tissue harvest be performed prior to breach of the skin's integrity. If the mass has abraded or is necrotic, the tissue collected will not be of good quality and may not be sterile.

9. The body cavity is opened using the sterile instruments.

Spleen harvest procedure:

To harvest the spleen, the body is positioned in right lateral recumbency. The spleen can be visualized through the abdominal muscle. A scissor cut is made directly over the spleen and it is gently retracted from the body cavity. Take care to avoid puncturing or tearing the organ as you cut all attachments between the spleen and the stomach and pancreatic tissue.

a. To harvest any other abdominal organs, the body is placed in dorsal recumbency. The abdominal muscle is lifted and cut away leaving an oval opening directly over the abdominal cavity. Organs are harvested in the following order to maintain sterility of the instruments.

Liver harvest procedure:

After exposing the abdominal cavity, reflect the liver caudally away from the diaphragm. Carefully sever any membranes attaching the liver to the diaphragm. Make a cut perpendicular to the spine through the blood vessels visible between the liver and the diaphragm. Lift the liver and reflect it toward the diaphragm to visualize the attachments beneath the liver. There will be thin membranes attaching the smaller liver lobes to the stomach and small intestine which will need to be disrupted. Grasp the fibrous node in the center of the liver on the underside and lift the organ to find all the attachments. Cut away the liver from the underlying organs and tissues. Note that liver is fragile and can be easily punctured resulting in release of blood, therefore it must be handled carefully.

Kidney/Adrenal gland harvest procedure:

After exposing the abdominal cavity, push the intestines to the left to visualize the right kidney or right to visualize the left kidney. Grasp beneath the kidney using the length of the forceps blades to isolate the organ from the body. Cut beneath the forceps to avoid damaging the kidney. Once the kidney is removed any fat can be trimmed from the organ. The adrenal glands are located just anterior to the kidneys, often in a fat deposit.

Ovary harvest procedure:

After exposing the abdominal cavity, move the intestines cranially to visualize the uterus. Follow the left and then the right horns of the uterus to the ovaries. The ovaries are small and in older animals may be imbedded in fat. Slide the forceps under the ovary to isolate it from the body and cut it free from the fat and fallopian tube.

Testes harvest procedure:

After opening the abdominal cavity, gently grasp the inguinal fat pad and pull anteriorly. This will pull the testis from the scrotum. Once the testis is exposed, cut the attachments to the scrotum and the vas deferens to free it from the body. This is done for each testis.

b. Other organs in the abdominal cavity are harvested in the following order to maintain sterility of the instruments:

- Accessory sex organs – prostate, seminal vesicles, fallopian tubes, uterus
- Pancreatic tissue
- Lymph nodes
- Urinary bladder
- Intestinal tract – stomach, small intestine, large intestine

c. To harvest thoracic cavity organs the body is placed in dorsal recumbency. It is imperative that the skin be reflected well above the head if thoracic cavity organs are to be harvested. Grasp the xiphoid process and lift to allow a cut through the abdominal muscle just caudal to the ribs. Extend the cut along the lower curve of the rib cage on both sides. This will allow visualization of the diaphragm. Puncture the diaphragm by making a small cut in the rib cage at the most lateral point possible on each side, being careful not to touch the scissors to non-sterile surfaces. Cut the diaphragm away from the ribs. Lifting the ribs at the xiphoid to visualize the thoracic organs, extend the lateral cut to the top of the sternum on both sides and open the chest.

Heart harvest procedure:

To remove only the heart, lift the heart and carefully cut through the pericardial sac. The aorta is at the cranial aspect of the heart on the animal's left. Holding the aorta with the forceps, make a cut distal to the forceps retaining hold on the aorta and heart. Grasp the aorta as you sever the anterior vena cava and pulmonary arteries and veins and the posterior vena cava. Lift the heart free of the thoracic cavity by the aortic artery.

Lung harvest procedure:

The heart and lungs are most easily removed together. Once removed, the heart can be excised from the lungs. Place the forceps perpendicular to the trachea and grasp the trachea firmly. Using the scissors, make a cut perpendicular to the trachea just anterior to the forceps. This cut should sever the trachea and esophagus. Without loosening the grip on the trachea, lift the trachea up and caudally and snip any attachments of the lungs to the spinal surface in the rib cage. The esophagus may need to be cut to be able to lift the heart and lungs free of the chest cavity.

d. To harvest any other thoracic organs, the body is placed in dorsal recumbency. The thoracic cavity is opened in the same manner as for the harvest of heart/lungs. Organs are harvested in the following order to maintain sterility of the instruments:

- Thymus
- Lymph nodes
- Esophagus

10. In special circumstances the brain will need to be harvested for sterile culture. The brain is harvested last after any other organ harvests.

Brain harvest procedure:

In instances where brain is the only organ to be collected, the skin is not stripped from the body but instead a skin cut is made at the nape of the neck and extended along the midline from the dorsal cervical area to the tip of the nose. After pulling the skin away from the skull laterally, the exposed bone is soaked with 70% alcohol and the soiled top gloves are removed. One hand will be considered non-sterile as it grasps the skin from the skull reflected beneath the chin to stabilize the head while the skull is cut and the brain removed. Using a dedicated pair of sterile brain harvest scissors, make a cut through the spine at the base of the skull. Open the skull by placing the point of the scissors in the foramen magnum and cutting along the midline. Use the flat of the scissor blade to lever the parietal bones away from the brain. Carefully disrupt the nerve attachments at the brain stem and the optic chiasm beneath the brain using the closed point of the thumb forceps. In a similar manner slide the closed forceps rostrally beneath the brain and break the attachments at the olfactory bulbs. The brain can then be dropped from the skull into sterile media.

11. New gloves will be needed for each mouse unless the harvests are done assembly line fashion where the same step is repeated on multiple animals culminating in all tissues excised as the final step.